1. Title of the thesis

"Synthesis and Characterization of Organic Phosphate Esters and their Hydrolysis"

2. Introduction

Phosphorus is one of the most abundant elements on earth. Most of the phosphorus in living systems exists in the form of phosphate. It is part of many essential biological components such as lipids, bones, genetic materials, energy rich molecules (e.g.-ATP) and signaling molecules (e.g.-GTP) [1]. Phosphate ester hydrolysis is a crucially important reaction in biological systems, being involved in biosynthesis processes. Organophosphate esters are the derivatives of orthophosphoric acid, which can in principle be esterified at any or all of three different positions, forming monoesters, diesters and triesters and the reaction mechanism will vary according to the system[2]. Organophosphates having C-N-P linkage is of great importance and display a significant role in various fields. In the recent years most of the studies have been concentrated on the mechanism of biochemical actions, stereochemistry, pharmacological actions, toxicological effects and decontamination aspects of organophosphorus compounds[3].

Organophosphate esters have wide range of applications in the industrial, agricultural and academic field owing to their unique biological and physicochemical properties. Due to these properties, they have used as reagent in organic synthesis[4]. Phosphate esters are important surfactants, which are widely used in many fields, such as chemical fibers, daily chemical products, plastic and paper-making[5]. They are also used as additives in the textile and clothing dyeing industry. Nucleoside phosphates and their phosphonate analogues have proven to be exceedingly important agents for anticancer, antiviral therapy[6] and act as HIV protease inhibitors[7]. Phosphonate containing drugs are increasingly being explored in other therapeutic areas[6]. The extreme toxicity, broad-spectrum activity and low cost of organophosphorus

compounds have made them popular as pesticides, insecticides, bactericides and antibiotics[8, 9].

Now a day's catalytic cleavage of organophosphate esters, detailed fundamental studies on nucleophiles aided hydrolysis and micellar catalysis are in progress. Surfactants generally form spherical micelles in aqueous solutions above the critical micelle concentration (CMC). The micelles can affect the rate of reactions by concentrating the reactants and providing a reaction environment that is different from that of the aqueous solution[10]. Micelles and other association colloids act as self-assembled micro reactors-compartmentalizing, concentrating or separating and diluting reactants, thereby altering rate equilibrium constants of chemical reactions[11].

In view of this, the study of hydrolysis of variety of organic phosphate has become the subject of research interest to chemist and biologist during the past few decades. The present work concentrates on synthesis of new phosphate esters and their hydrolytic studies under different experimental conditions. The use of novel surfactants in assisting a variety of organic reactions is highly promising for basic and applied research. Hence the work will be further extended to study the effect of micelles on progress of the hydrolytic reactions of organic phosphate esters, using different surfactants.

3. A brief review of the work already done in the field

The multidimensional importance of organophosphate esters leads to vigorous research developments, which have been reviewed as given below-

Comparative studies on reaction of bis (p-nitro phenyl) phosphate and α-nucleophiles in cationic micellar media have been studied by Ghosh et al.[12] (2012). They reported the cleavage of bis (p-nitro phenyl) phosphate (BNPP) over a pH range of 7.0-12.0 in the presence of different α- nucleophiles. Rajanna and coworkers[13] (2011) have carried out kinetic and mechanistic study of hydrolysis of thiamine

pyrophosphate (cocarboxylase) in aqueous buffer and micellar media. Ashkenazi et al.[14] (2010) have reported the mechanism of nucleophilic displacements at phosphorus in chloro-substituted methylphosphonate esters. They have also studied P-O V_S P-C bond cleavage by using density functional theory (DFT).

Singh and Shinde[15] (2009) have carried out the kinetics and mechanism of hydrolysis of Di-2-chloro-5-nitro aniline phosphate in acid region 0.1 to 7.0 mol dm^{-3} HCl at 80^oC in 10% aqueous dioxane (v/v) medium. Klugar and wodzinska[16] (2008) have studied the pK_a-dependent formation of amides in water from an acyl phosphate monoester and amines. Kim et al.[17] (2008) have carried out the structure of diethyl phosphate bound to the binuclear metal center of phosphotriesterase. They have also calculated density functional theory, which supported a reaction mechanism.

Kamerlin and Wilkie[18] (2007) investigated the impact of metal ion on the potential energy surface and hence preferred reaction mechanism for simple model for hydrolysis of phosphate ester mono anion. Lopez et al.[19] (2006) have carried out a density functional study of the hydrolysis reaction of phosphodiesters with a series of attacking nucleophiles in the gas phase and in solution. Vigroux and coworkers[20] (2005) have employed DFT calculations and dielectric continuum methods, to map out the lowest activation free-energy profiles for the alkaline hydrolysis of phosphate triesters and diesters.

Micellar solutions of sulfobetaine surfactants in water-ethylene glycol mixtures have been studied by Moya et al.[21] (2005). They have explained quantitatively kinetic micellar effects by using the pseudo phase kinetic model. Bhattacharya and Kumar[22] (2005) have studied ester cleavage properties of synthetic hydroxybenzotriazoles in cationic monovalent and Gemini surfactant micelles. Hengge et al.[23] (2003) have carried out the study of hydrolysis reactions of alkyl versus aryl phosphate monoester monoanions. They proposed a mechanism in which the phosphoryl group deprotonates water and then undergoes attack by hydroxide. Dejaegere and coworkers[24] (2002)

have studied theoretical evaluation of pK_a in phosphoranes. They calculated pK_a's of the model phosphorane, ethylene phosphorane and employed density functional theory to calculate the gas-phase protonation energies.

Mechanistic alternatives in phosphate monoester hydrolysis have been proposed by Aqvist et al.[25] (1999). They have reported a closer thermodynamic analysis of observed linear free energy relationships, which have provided experimental information that is consistent with the associative, concerted and dissociative alternatives. Ionescu and Souza[26] (1998) have studied micellar catalyzed reactions of a phosphate ester. The hydrolysis of p-nitrophenyl diphenyl phosphate has also studied in aqueous solutions containing micelles of cetyltrimethylammonium bromide (CTAB), sodium hydroxide and various concentrations of dimethylsulfoxide (DMSO).

4. Noteworthy contribution in the field of proposed work

Organophosphorus compounds are very important, due to their wide range of applications in various fields. These compounds manufactured by the chemical industry consist of arylated and alkylated phosphates such as phosphites, phosphines and related dimeric, ionic forms and utilized widely. Tris(2-chloroisopropyl) phosphate (TCPP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloro-1-(chloromethyl)ethyl) phosphate (TDCP), tris (2-butoxyethyl) phosphate (TBEP), and triphenyl phosphate (TPP) are mainly used in variety of products, such as plastic materials, varnishes, lubricants, hydraulic fluids[27]. Metallocomplexes of dithiophosphate diesters have been employed for many years as lubrication components in many applications including automobile engines[28].

Phosphorus compounds are also important in foodstuffs for example, phosphoric acid acidifies soft drinks[29], Sodium pyrophosphates in baking mixtures as acidulants and in potato processing[30]. Organophosphorus compounds are a diverse

group of chemicals used in both domestic and industrial setting. Examples of organophosphates include antihelmintics (trichlorfon), and ophthalmic agents (echothiophate, isoflurophate). Herbicides [tribufos, (DEF)] are tricresyl phosphate containing industrial chemicals[31]. Trisubstituted phosphorus acid (tri aryl or trialkyl phosphate) such as triphenyl phosphate and tri-iso-propyl phosphate used as an antioxidant while S-S-S, tri n-butyl phosphorotrithioite (Merphos) is used as a cotton defoliants[32]. These compounds are used in pesticides sprayed and dusted onto cereals, fruits and vegetables. P- Nitrophenyl substituent, such as methyl parathion, parathion, diazinon, ethion and fenthion[31] are extensively used as agriculture pesticides in the United States and around the world[33]. Phosphorus being a part of important biomolecules plays a fundamental role in microbial cell physiology and biochemistry[34]. Some of the benzoxazaphosphinines posses significant microbial activity[35].

5. Proposed methodology during the tenure of research work

New organic phosphate esters will be synthesized by the known methods described in earlier literature[36] which involves the reaction of parent compound with phosphorylating agents such as $POCl_3$, PCl_5 and P_2O_5 in different ratios. Confirmation of the new organic phosphate esters will be done by following steps:-

1. Melting point of the compounds will be noted.
2. Elemental analysis of compounds (C, H, N & P) will be done.
3. IR spectra will be recorded.

After confirmation of the compounds, Kinetic study of the hydrolysis of these phosphate esters will be done under different experimental conditions as given below:-

1. Hydrogen ion concentration
2. Ionic strength
3. Temperature effect
4. Solvent effect

5. Substrate concentration.

The progress of hydrolytic study of phosphate esters will be studied by Allen's modified method[37] using spectrophotometer. The inorganic phosphate produced during hydrolysis forms a phosphomolybdate complex which reduces to molybdenum blue by the action of mixture of amidol and sodium metabisulphite solution. The intensity of the blue colour is proportional to the amount of free phosphoric acid.

CMC determination is an important physicochemical exercise for self-assembly surfactant solution. The CMC determination will be carried out by the most frequently used methods as surface tension[38], conductometry[39] etc.

6. Expected outcome of the proposed work

The mono, di, tri, phosphate esters will be obtained with many significant properties, useful in various fields of chemistry such as medicinal, agricultural and industrial. Kinetic study of the hydrolysis of these phosphate esters will provide information about the mechanism. The determination of mechanism will give the knowledge of different possible reaction paths and may provide substantial basis for understanding the reactions of complicated phosphate esters. The study of the mechanism is of equal interest for both physico-organic chemists as well as bio-chemists. The structure and properties of surfactants play an important role in determination of chemical reactivity. Therefore, kinetic study of phosphate esters with micelles will provide a green catalyst with excellent rate enhancement as well as do significant contribution in various fields of chemistry.

7. Bibliography:

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